

**HETEROLOGOUS RECOMBINATION BETWEEN
MOUSE MYELOMA 315 HEAVY CHAINS
AND RABBIT ANTIBODY LIGHT CHAINS :
STRUCTURAL AND FUNCTIONAL PROPERTIES
OF THE HYBRID MOLECULES**

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présentée à la Faculté de médecine de l'Université de Genève
pour obtenir le grade de docteur en médecine

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La Faculté de médecine, sur le préavis du professeur Pierre Vassali, autorise l'impression de la présente thèse, sans prétendre par là émettre d'opinion sur les propositions qui y sont énoncées.

Genève, le 7 juin 1974.

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ABSTRACT

7S recombinant molecules have been obtained from the heavy chains of mouse myeloma protein 315 (which binds Dnp ligands) and the light chains from rabbit anti-Dnp antibodies or normal rabbit IgG. Heavy and light chain antigenic determinants were detectable on these recombinants, and they displayed structural properties that closely resembled those of the parent molecules with respect to sedimentation coefficient, heavy to light chain ratio and gel filtration characteristics. Recombinants containing light chains from anti-Dnp antibodies showed significantly greater binding of ϵ -Dnp-lysine than hybrids made with nonspecific light chains. Idiotypic determinants characteristic of the intact protein 315 were not recovered on any of these recombinants to any greater extent than were expressed on 315 heavy chains alone. These results indicate that a Dnp binding site structure can be obtained by interaction between immunoglobulin subunits from different species, provided that they originate from anti-Dnp molecules. Also, these data indicate that monoclonal heavy chains reconstituted with polyclonal light chains display binding properties resembling a polyclonal (heterogeneous) antibody population.



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Abbreviations used in this work: Dnp, the 2,4-dinitrophenyl group; HSA, human serum albumin; PBS, phosphate buffered saline (containing 0.15 M NaCl 0.02 M potassium phosphate, pH 7.4); Dnp-HSA and Dnp-Hcy refer to heavily dinitrophenylated human serum albumin and Limulus polyphemus hemocyanin, respectively; SDS, sodium dodecyl sulfate; RDE and RDL refer to rabbit anti-Dnp antibodies isolated from pooled sera obtained early (RDE, 14 days) or late (RDL, 49 days) after immunization with Dnp-hemocyanin; the symbols HL^N , HL^{DE} , and HL^{DL} refer to recombinant molecules prepared from heavy chains from the IgA murine myeloma protein, 315, and light chains from nonimmune rabbit IgG (HL^N) or from rabbit anti-Dnp antibodies isolated from sera obtained early (HL^{DE}) or late (HL^{DL}) after immunization.

INTRODUCTION

Structural studies of immunoglobulins have shown that the heavy and light chain subunits can be separated in dissociating solvents after mild reduction and alkylation of the interchain disulfide bonds (1). Separated chains will recombine during dialysis against neutral aqueous solvents to yield four chain molecules with physical and antigenic characteristics resembling the parent molecules (2-8).

Reconstituted heterogeneous antibody populations fail to bind their specific ligand to the same extent as unseparated molecules. On the other hand, reconstituted molecules from monoclonal antihapten proteins (9-11) or from antibodies with restricted heterogeneity (12) have been shown to recover full binding activity.

We examine here an intermediate situation where heavy chains from the mouse myeloma protein 315 (which binds 2,4-dinitrophenyl (Dnp) ¹ ligands specifically) were reconstituted with light chains from conventionally induced rabbit anti-Dnp antibodies obtained early or late after immunization (13), or with light chains from nonspecific rabbit immunoglobulin G (IgG). Structural as well as functional characteristics of these hybrid molecules were investigated in an attempt to establish the capacity of heavy and light chains from different species to interact with the resultant expression of a Dnp specific ligand binding site. We have also evaluated the dispersion of intrinsic affinities exhibited by the active sites formed from heterogeneous light chains and monoclonal heavy chains derived from immunoglobulins of the same specificity.

MATERIALS AND METHODS

Plasmacytoma and Protein Purity. MOPC 315 was maintained by transplantation of subcutaneous tumors in BALB/c AnN mice. Protein 315 was purified from pooled sera by immunoadsorption at 25° C on a dinitrophenylated human serum albumin (Dnp-HSA) polyacrylamide column (14), followed by specific elution with 0.1 M Dnp-glycine in 0.2 M Tris-Cl buffer pH 8.0. The eluted protein was then exhaustively dialyzed against phosphate buffered saline (PBS) at 0-4° C before concentration by ultra-filtration. The protein concentrate was made 0.2 M Tris-Cl pH 8.0 by addition of 1 M buffer, reduced with dithiothreitol (P-L Biochemicals, Milwaukee, Wis.) (final concentration 0.01 M) for 2 hrs at 25° C, and alkylated for 15 min. at 25° C with a twofold molar excess of iodoacetamide (Sigma Chemicals, St. Louis, Mo.) with respect to the thiol reagent. The reaction was stopped by dialysis against PBS at 0-4° C. The reduced and alkylated protein was further purified by G-150 Sephadex gel filtration (two 2.5 x 80 cm columns run in tandem) in PBS at 0-4° C and the 10 % of the applied proteins emerging at the void volume was discarded. The slightly retarded main peak was pooled and checked for purity by three criteria: (a) By immunoelectrophoresis, the purified protein showed a single arc when reacted with a goat antiserum made against the globulin fraction of serum from mice bearing the MOPC 315 plasmacytoma. (b) By fluorescence quenching at 4° C using Dnp-lysine as ligand, 60 to 65 % of the protein fluorescence was quenched. (c) The A360nm/A278nm ratio of the

protein did not exceed 3 %, making any further hapten removal step unnecessary. The purity of protein 315 was comparable to that obtained in previous studies (9,15).

Isolation of Rabbit Anti-Dnp Antibodies. 10 Albino rabbits (Pel-Freez, Rogers, Arkansas) were each injected in the footpads with a total of 1 mg of highly substituted Dnp-hemocyanin (Dnp-Hcy) emulsified in complete Freund's adjuvant and bled 2 weeks later to yield an early anti-Dnp pooled antiserum (RDE). Quantitative precipitin analysis of the RDE pool with highly substituted Dnp-HSA revealed a concentration of 1.12 mg/ml of anti-Dnp antibody. The rabbits were boosted 4 weeks after the first injection with the same amount of Dnp-Hcy in complete Freund's adjuvant in the same sites. A second bleeding 3 weeks after the boost yielded a late anti-Dnp pooled antiserum (RDL) containing 6.5 mg/ml of antibody.

Both antisera were purified by adsorption on a Dnp-HSA polyacrylamide column (14) at 25° C. The antibody was eluted with 0.1 M Dnp-glycine in 0.2 M Tris Cl pH 8.0 and exhaustively dialyzed against PBS at 0-4° C. Residual hapten was removed by passage through a column of Dowex AG-1 x 8 (200-400 mesh) equilibrated with PBS. 10-15 % of RDE sites and 30 % of RDL sites were still occupied by hapten after the Dowex purification step, as determined by A360nm/A278nm absorbance measurements.

All preparations of rabbit antibodies or nonimmune IgG gave a single arc in immunoelectrophoresis when run at 5-10 mg/ml and developed with a goat antiserum against rabbit whole serum. Antibodies were stored in lyophilized form. Rabbit IgG was purchased from Pentex, Kankakee, Ill. (lot 68) and used without further purification.

Chain Separation. The 315 protein contained in the main Sephadex G-150 peak was reduced and alkylated a second time (same conditions as before) to ensure the subsequent complete separation into heavy and light chains. Chain separation was achieved at 0-4° C by filtration of 20 mg of twice reduced and alkylated 315 protein on a 2.5 x 80 cm G-100 Sephadex column in 4.5 M urea (previously deionized with Amberlite MB-3) and 1 M propionic acid (distilled before use). The purity of 315 H and antibody L chains was checked by polyacrylamide disc gel electrophoresis in 1 % sodium dodecyl sulfate (SDS) (16).

Isolated anti-Dnp antibodies in 0.5 M Tris buffer, pH 8.0 were reduced only once with 0.1 M 2-mercaptoethanol (Sigma) for 1 hr at 25° C and alkylated 1 hr at 25° C with iodoacetamide in 10 % molar excess with respect to the thiol reagent. Separation of chains was achieved in propionic acid and urea on the same G-100 Sephadex column employed for separation of H and L chains of protein 315.

Recombination Procedure. The recombination was carried out according to Bridges and Little (9), modified as follows: Approximately 16 mg of total immunoglobulin chains were mixed in an absorbance ratio of 1:2 light chain to heavy chain (approximately 1.5L to 1H molar ratio). HSA (from which aggregates had been removed by passage over G-150 Sephadex columns) was added to the recombination mixture to a final concentration of 0.5 mg/ml. Other parameters of the procedure are shown in Table I (see below). Renatured protein solutions were concentrated

by ultrafiltration on Amicon PM 10 membranes before gel filtration on two tandem columns of G-150 Sephadex (2.5 x 80 cm each) in PBS, .05 %NaN₃, at 4° C. Representative elution profiles are illustrated below in Fig. 1. Recombinant molecules free of aggregates were pooled and concentrated for further analysis.

Anti-Idiotypic Assay. The expression of 315 idiotype on various immunoglobulins was determined by the extent to which they inhibited the reaction between ¹²⁵I labeled 315-protein and an anti-idiotypic antiserum raised in BALB/c AnN mice, according to the immunization schedule of Sirisinha and Eisen (17). The goat anti-mouse Ig (Gateway Immunosera, Cahokia, Ill.) used to precipitate the primary immune complex was absorbed before use on a column of Sepharose immunoabsorbant coupled to 315-protein by the CNBr method (18). Dried precipitates were counted in a Nuclear Chicago well-type gamma counter. 315-protein was labeled with Na ¹²⁵I by the method of Sprada and Schlamowitz (19).

Equilibrium Dialysis. Equilibrium dialysis was performed in lucite chambers (0.1 ml capacity) with 50 μ l of test protein separated by dialysis casing from 50 μ l of (³H)- ϵ -Dnp-L-lysine at various concentrations. An amount of normal rabbit Ig equal to that of the test protein was introduced into the hapten side to minimize Donnan effects. Control chambers, containing equivalent concentrations of rabbit IgG on both sides of the membrane, were used to determine that hapten equilibrium had been attained. Chambers were left at 0-4° C for 80 to 90 hrs without agitation. At equilibrium, 5 μ l was sampled from both sides of the dialysis membrane using Drummond capillary pipets, and dissolved in 10 ml aliquots of Bray's solution (20). Radioactivity was counted on a Packard scintillation spectrometer equipped with an automatic external standard.

Average intrinsic association constants, K_O, were calculated as described (21). In the notation employed, $\bar{r}$ is moles of hapten bound per mole of immunoglobulin, $\bar{n}$ is the limiting value of $\bar{r}$, c is the molar free hapten concentration, and a is the Sips heterogeneity index (22).

Miscellaneous. Extensively reduced and alkylated protein samples were analyzed by 1 % SDS disc gel electrophoresis, as described by Laemmli (16). Gels were fixed in hot 7.5 % TCA and stained with Coomassie blue. Densitometer tracings of stained gels were recorded at 600 nm on a Gilford 240 spectrophotometer equipped with a gel scanner. From these tracings, H to L ratios were estimated by triangulation and by weighing cut peak areas (see below Table I).

Sedimentation coefficients of protein samples at 0.5 mg/ml in PBS were measured at 20° C, 60,000 rpm, in a Beckman Model E analytical ultracentrifuge equipped with ultraviolet absorbance optics.

(³H)- ϵ -Dnp-L-lysine was synthesized as described by Eisen et al. (23), and the purity was established by thin-layer silica gel chromatography in water-saturated methyl-ethyl-ketone, by comparison of the absorbance spectrum to that of authentic ϵ -Dnp-L-lysine, and by equilibrium dialysis with a purified rabbit antibody specific for the dinitrophenyl group, in which case the distribution of ligand between the compartments was the same whether measured by absorbance (360 nm) or by radioactivity.

Fluorescence quenching measurements (24, 25) were obtained with an Aminco-Bowman spectrofluorometer.

Protein concentrations were measured with a PMQ-II Zeiss spectrophotometer, using $E_{278}^{1\%}$ of 15 for 315 protein, rabbit antibodies and normal rabbit IgG. The same extinction coefficient was used for recombinants.

RESULTS

Efficiency of Chain Separation. It was found necessary to reduce and alkylate 315 protein twice to ensure quantitative separation into heavy and light chains. After a single reduction and alkylation, 315 heavy chain preparations contained 4 to 8 moles percent contaminating light chains as detected by SDS disc gel electrophoresis and comparison with graded amounts of light chains run in parallel in the same gel system. Completely reduced and alkylated heavy chains from twice reduced and alkylated 315 protein contained less than 1 mole percent light chain.

Heavy and light chains from rabbit antibody molecules were also examined by SDS disc gel electrophoresis after complete reduction and alkylation. Cross contamination was not found under conditions where greater than 1 % contaminants would have been detected.

Efficiency of Recombination. 315 Heavy chains mixed with light chains from antibodies or from normal IgG yielded 7S recombinants with approximately equal efficiency. Representative gel filtration elution profiles of the 7S recombinants are illustrated in Fig. 1. The efficiency of 315 heavy chain and rabbit light chain recombination is as good as in homologous 315 protein recombination experiments (9). Table I summarizes the physical properties, yields, and heavy and light chain composition of various recombinants. 315 Heavy chains were reproducibly stabilized by light chains to form four chain structures, as evidenced by their position in gel filtration profiles and by their sedimentation coefficients. Recombinants of each chain combination were prepared twice and comparison of physical parameters within each set was satisfactory.

The methods used to estimate the light and heavy chain composition of the recombinants permit us to state that the ratio is near unity, and compares well with values obtained for the parent molecules (See Fig. 2 and Table I). On immunoelectrophoresis (Fig. 3), the rabbit light chain arc and the 315 heavy chain arc fused into one single football-shaped precipitin line, demonstrating the presence of the two types of antigenic determinants on the same molecule, whose electrophoretic mobility corresponds to that of mildly reduced and alkylated 315 protein.

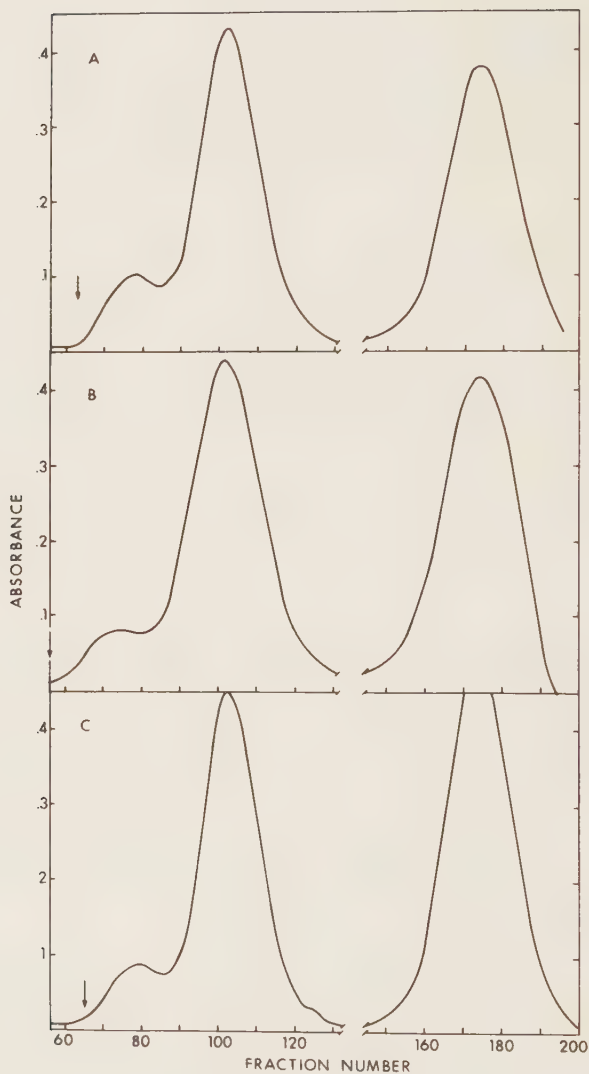


Figure 1.

Gel filtration elution profiles. Separation of recombinant species from aggregates and from HSA was obtained by gel filtration on Sephadex G-150 in PBS and .05 % NaN_3 . Two columns (2.5 x 80 cm each) were connected in tandem and the void volume was determined by blue dextran (Pharmacia). The first peak was composed of aggregates containing heavy and light chains; the second peak was the 7S recombinant species. The HSA was recovered quantitatively as a third major elution peak. No free light chain was detected in these experiments but a fourfold molar excess of light chains was seen when heavy chains were mixed with a twofold molar excess of light chains. The three profiles illustrate results with recombinants $\text{HL}^{\text{NII}}(\text{A})$, $\text{HL}^{\text{DEII}}(\text{B})$, and $\text{HL}^{\text{DLII}}(\text{C})$. The ordinate for each profile is 278 nm absorbance.

TABLE I

Summary of Recombination Experiments and Properties of the Recombinants

Recombinant Designation	Recombination Mixture ^a		Yield of 7S Recombinant from G-150 ^b	V _e /V _o ^c	S _{20'} ^w	Percentage of L Chain in Recombinants	
	Ig Concentration	L/H Molar Ratio				Triangulation ^d	Weight ^e
	(mg/ml)		(%)			(%)	(%)
HL ^N I	0.230	1.44	80	1.20	nd	36	32
HL ^N II	0.260	1.50	73	1.27	6.23	nd	
HL ^{DE} I	0.220	1.50	68	1.27	nd	42	36
HL ^{DE} II	0.242	1.50	78	1.26	5.9	nd	
HL ^{DL} I	0.200	1.50	80	1.23	nd	36	33
HL ^{DL} II	0.230	1.50	80	1.23	6.13	nd	
315 RA				1.23-1.27		41 -	37 ^f
RDL						32	36 ^f

^a All recombination mixtures were made in a solution containing 0.5 mg/ml of HSA.^b Percentage immunoglobulin absorbancy eluted from the column as a 7S peak over the total absorbancy included in the 7S peak and the aggregate peaks. 9 to 11.5 mg of 7S recombinants were usually recovered for a 16 mg total immunoglobulin input. $\text{CV}_e/\text{V}_o = \frac{\text{elution volume of 7S recombinant}}{\text{void volume determined by blue dextran}}$ ^{d, e} Each peak on the densitometer tracing was estimated either by d) calculation of the triangular surface that best fit its total area, or e) weighing each area cut out of the Gilford chart paper. Each was expressed as percent of total surface or weight accounted for by the light chain peak in each recombinant molecule.^f Values were obtained for these proteins prior to dissociation and chain separation.

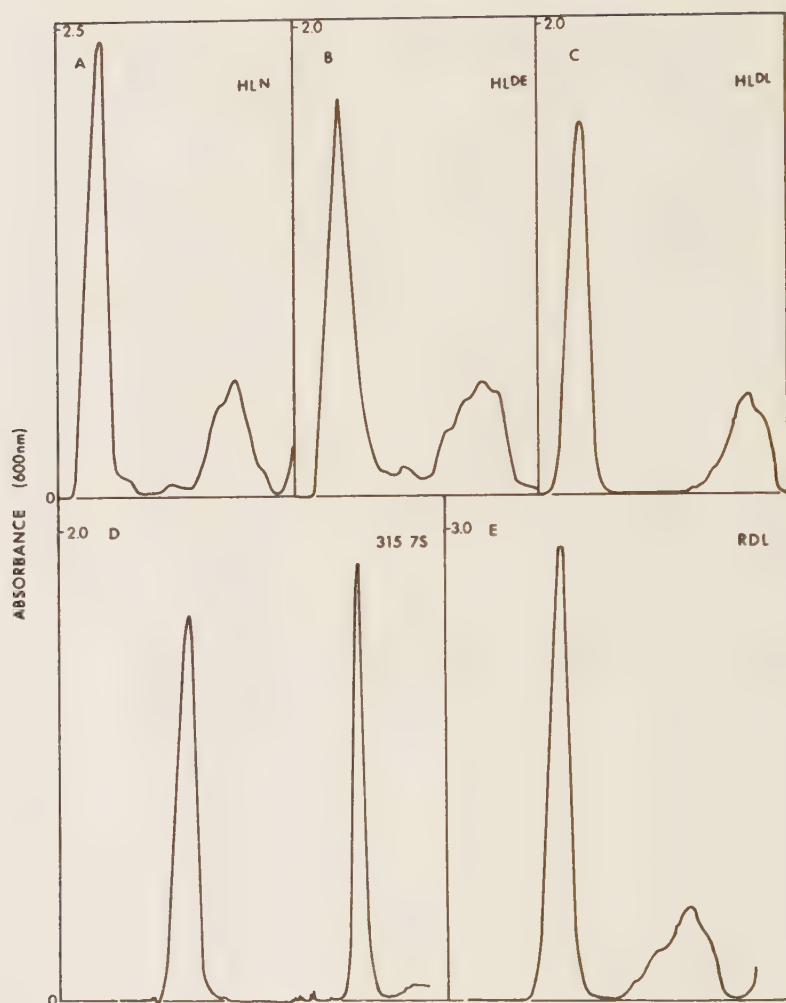


Figure 2.

Densitometer tracings of SDS acrylamide gels. Coomassie blue stained gels were scanned at 600 nm on a Gilford 240 spectrophotometer equipped with an automatic gel scanner. Migration is from the left to the right. The right end of each tracing corresponds to the position of the tracking dye (bromophenol blue). Calculation of relative peak areas was done by weighing the cut paper profile from densitometer tracings and by triangulation (see Table I). The mobility of rabbit γ -chains was consistently greater than that of 315- α -chains and homogeneous 315 light chains clearly had a narrower banding pattern than rabbit light chains.

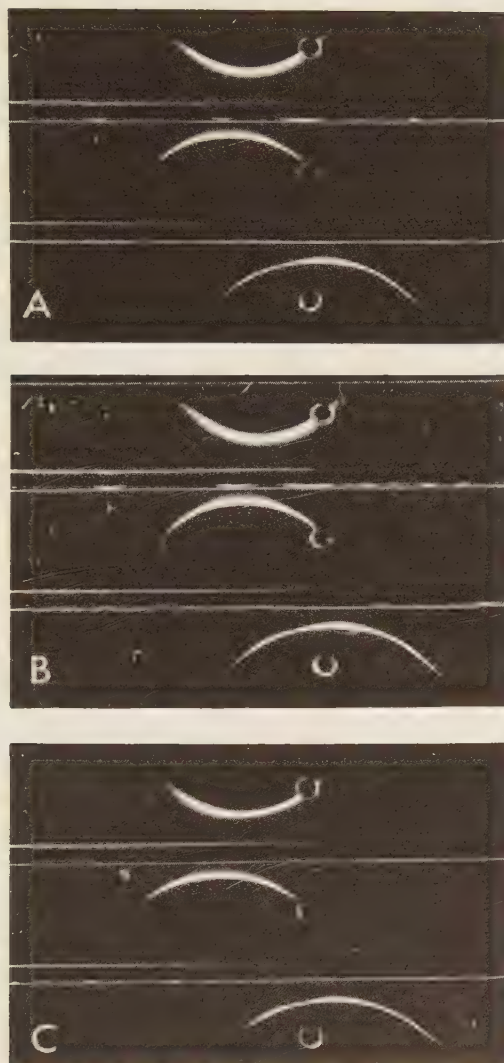


Figure 3.

Immunoelectrophoresis. The cathode is to the right. The gels are 0.8 % agarose in Na barbital buffer, $\Gamma/2 = .04$, pH 8.2. Electrophoresis at 240 V for 2 hrs. All protein samples: 5 mg/ml in PBS. For A, B and C: upper trough contains a goat antiserum monospecific for 315 heavy chains. The lower trough contains a goat anti-rabbit IgG antiserum that reacts with both H and L chains. 315 Protein was in each upper well.

A. Middle well: HL^N , lower well: rabbit IgG.

B. Middle well: HL^{DE} , lower well: RDE.

C. Middle well: HL^{DL} , lower well: RDL.

Control experiments were performed by mixing 315 heavy chains with HSA alone. The elution profile of such renatured immunoglobulin chains on Sephadex G-150 columns showed the presence of approximately equal amounts of aggregate and of a species characterized by a V_e/V_o of 1.32 to 1.34. Pooled unaggregated material, which was concentrated by ultrafiltration, gave symmetrical and sharp sedimentation boundaries in the ultracentrifuge implying molecular homogeneity but the sedimentation coefficients of several preparations varied from 5.7 to 6.7S indicating that the number of heavy chains per renatured species was not constant. Repeated sedimentation experiments also showed that the renatured heavy chains became aggregated within a week as indicated by increasing values of $S_{20,w}$ and the development of unsymmetrical boundaries.

315 Idiotypic Determinants. 315 Idiotypic determinants were present on recombinants to the same extent as on 315 heavy chains alone, but 100 to 200-fold greater amounts were needed to achieve the same 50 % inhibition as $1\mu\text{g}$ of mildly reduced and alkylated 315 protein (Fig. 4).

Ligand Binding Properties of Reconstituted Molecules. The amount of ligand binding exhibited by recombinants was considerably less than that of the parent molecules. However, significant differences existed between recombinants made with normal rabbit light chains and recombinants containing light chains from anti-Dnp antibodies.

Two sets of equilibrium dialysis experiments were performed and the data have been calculated as described by Nisonoff and Pressman (21). In the first set of experiments, HL^{N} I, HL^{DE} I and HL^{DL} I were tested for binding against 3 different concentrations of ϵ -Dnp-lysine (Fig. 5A). For HL^{N} I, only at the lowest hapten concentration tested was there a significant difference in the ligand concentrations across the dialysis membrane, which represented an average r value of 0.02. This is very similar to the value obtained with a preparation of renatured H chains of protein 315 (Fig. 5A). With HL^{DE} I and HL^{DL} I, on the other hand, the differences were significant for all hapten concentrations tested. The HL^{DL} I binding curve showed greater r and r/c values than did the HL^{DE} I curve. Both these curves were indicative of a heterogeneous type of binding.

To obtain more detailed information, equilibrium dialysis experiments were repeated with recombinants made from freshly prepared aliquots of the same polypeptide chains. Five hapten concentrations were then tested (Fig. 5B). HL^{N} II bound minimal ligand with a maximum r value of .018. For HL^{DE} II and HL^{DL} II, r values were measurable for all hapten concentrations. When plotted on Scatchard coordinates (Fig. 5B), an extrapolated n value of 0.15 was estimated. The experimental points were then plotted on Sips coordinates assuming an n value of 0.15 and a regression line was drawn by the least squares method. The heterogeneity index for HL^{DE} II was 0.58, and for HL^{DL} II was 0.29. Even if .10 were chosen as an n value, the heterogeneity indices were .73 and .71 for HL^{DE} II and HL^{DL} II, respectively. Therefore these data indicate a heterogeneity of intrinsic association constants of recombinant molecules that is comparable to conventionally induced rabbit anti-Dnp antibodies (13).

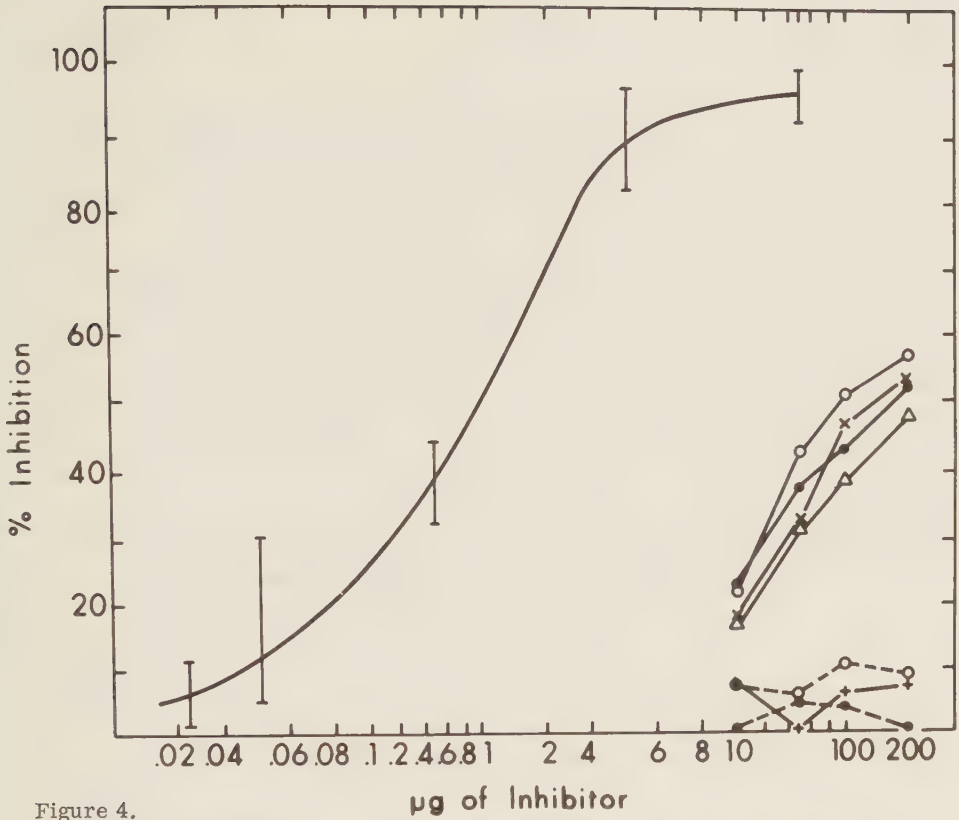


Figure 4.

Inhibition of ^{125}I trace labeled 315 protein binding to an antiserum specific for the 315 idiotype. Various amounts of recombinants or other immunoglobulins (as inhibitors) were incubated with 25 μl of anti-idiotypic antiserum for 30 min. at 24°C . 5 ng of ^{125}I 315 was then added and incubated for 15 min. at 37°C . 100 μl of goat anti-mouse Ig was added, and the tubes remained overnight at 4°C . Precipitates were washed, dried and counted in a well-type counter. I—I Mildly reduced and alkylated 315 protein, $\pm$ one standard deviation.

0—0 HL^{N} I

●—● HL^{DL} I

△—△ HL^{DE} I

X—X Separately renatured H chains from 315 protein.

○—○ RDE

●—● Rabbit IgG

+—+ RDL

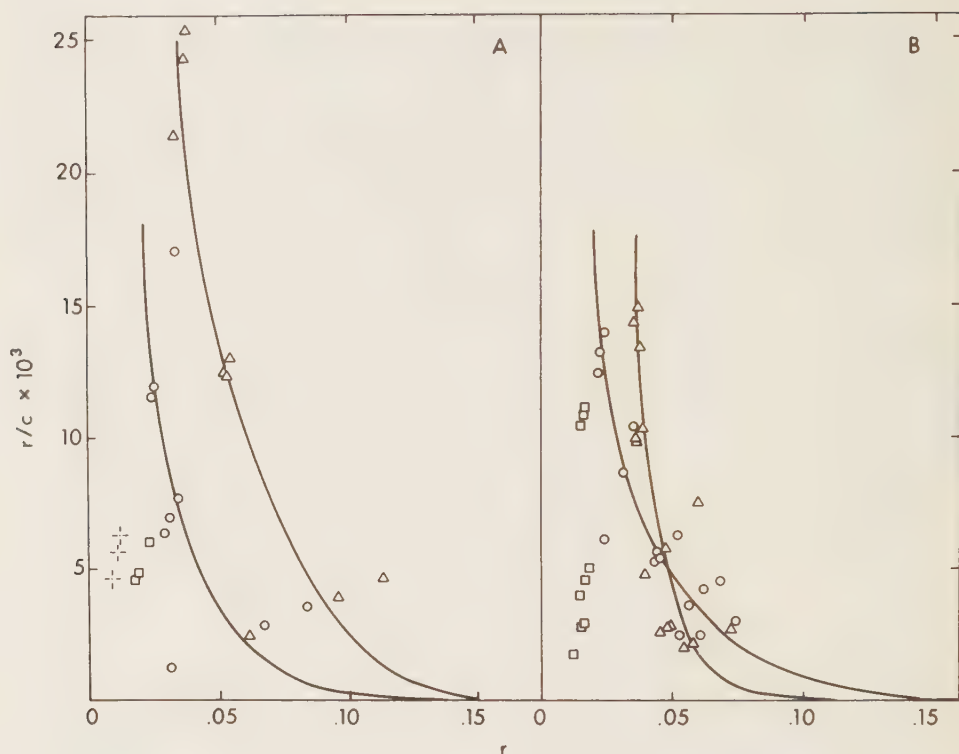


Figure 5.

Binding of (^3H)- α -Dnp-L-lysine by HL^{N} I and II, HL^{DE} I and II, and HL^{DL} I and II determined by equilibrium dialysis. The points on the figure represent individual experimental points. A regression line was calculated by least square analysis for each set of experimental points on Sips coordinates (not shown) assuming an n value of .15. r and c values for selected points on the regression line were calculated and plotted on Scatchard coordinates as the binding curve shown above. Protein concentrations: HL^{N} I 2.97×10^{-5} M; HL^{DE} I 2.88×10^{-5} M, HL^{DE} II 3.30×10^{-5} M, HL^{RL} I 2.84×10^{-5} M, HL^{RL} II 3.37×10^{-5} M.

A: HL^{N} I $\square$; HL^{DE} I $\circ$; HL^{DL} I $\triangle$; H_2 315 +

B: HL^{N} II $\square$; HL^{DE} II $\circ$; HL^{DL} II $\triangle$

DISCUSSION

Immunoglobulin polypeptide chain recombination provides a means of assessing the role played by heavy and light chains in the formation of antibody active sites. The use of a monoclonal heavy chain permits the analysis of a population of ligand binding sites to which the heavy chain contribution is a constant parameter. In order to compare the binding of recombinants containing different populations of polyclonal light chains, it is important to establish that their overall physical structure and polypeptide chain composition are analogous to conventional immunoglobulins. The data obtained here indicate that the recombinants tested have molar heavy and light chain ratios close to unity, and the sedimentation values, gel filtration and antigenic properties fall within a narrow range of anticipated values consistent with other four-chain immunoglobulin molecules. Therefore, comparisons of ligand binding properties can be reasonably considered to reflect the intrinsic contribution of the light chain to the newly formed active site.

A specific enhancement of ϵ -Dnp-L-lysine binding has been achieved for all recombinants containing light chains from anti-Dnp antibodies. In contrast, normal light chain containing recombinants did not display a significant increase in binding over the range of hapten concentrations tested. The r values of .015-.020 obtained for HL^N I and II probably represent binding by portions of the recombinant molecules other than the active sites.

In the HL^D experiments, binding curves extrapolated to r values in the range of .10-15. It seems unlikely that a value of 2 sites per molecule could have been attained; more likely, an r value of .15 expresses the maximum number of sites available for ϵ -Dnp-lysine binding. The polyclonal nature of an L^D population yields a variety of physically differing light chains whose capacity to interact with the same heavy chain will differ accordingly. It is consistent with results from ours and other laboratories (2-5, 8-11) that the structural requirements for reconstitution of a functional ligand binding site are much more rigorous than the requirements to form a stable four-chain molecule. However, it is significant that enhanced ligand binding was associated consistently with recombinant molecules whose light chains were derived from anti-Dnp antibodies. The corollary interpretation is that in each preparation bearing a "Dnp-specific" homogeneous heavy chain and heterogeneous light chains the majority of recombinant molecules' active sites had some alternative structure and, therefore, a different specificity. This is in accord with the presumption that a large number of different antibody specificities could result from a limited repertoire of light and heavy chain V genes.

It is noteworthy that our data show readily appreciable heterogeneity, despite the very low affinity of these recombinants. One would have expected light chains from the RDL antibody preparation to be of overall higher affinity than RDE light chains (13), and consequently enhance the recombinants' binding to a greater degree. In the first series of experiments (Fig. 5A), HL^{DL} I bound clearly more than HL^{DE} I, whereas in experiments represented in Fig. 5B no clearcut difference between HL^{DL} and HL^{DE} could be demonstrated.

The extent of inhibition in idiotype studies exhibited by all recombinants was in the same range (or less) as that reported by others for isolated 315 heavy chain (17, 27, 28), in comparison with mildly reduced and alkylated 315 protein. This is a good indication that our recombinants are free of contaminating 315 intact sites, or at least, contaminated at an equally minimal level. Failure to detect 315 idiotypic antigenic determinants on recombinants to any extent greater than on isolated heavy chains supports the view that the new binding sites formed exhibited significant differences in fine structure from the active site of the native 315 protein.

Results of the present studies are in agreement with reports indicating that heavy and light chain contributions to the active site are additive (29, 30) and that light chains contribute contact amino acids to the active site, as judged by affinity labeling experiments (31). Also, our results confirm (32-34) the findings that light chains from anti-Dnp IgG molecules have the capacity to combine with an anti-Dnp heavy chain of another class, with recovery of specific binding. Apart from protein solubility requirements, very few restrictions are imposed upon interaction of completely unrelated heavy and light chains. Their V_H and V_L domains show enough homologies (35, for review) to allow the formation of a uniform four-chain Ig recombinant stabilized by noncovalent bonds.

REFERENCES

1. Fleischman, J.B., Pain, R.H., and Porter, R.R., Arch. Biochem. Biophys., 1 (Suppl.) : 174, 1962.
2. Fougereau, M., Olins, D.E., and Edelman, G.M., J. Exp. Med., 120:349, 1964.
3. Hong, R., and Nisonoff, A., J. Immunol., 96:622, 1966.
4. Roholt, O., Radzimski, G., and Pressman, D., J. Exp. Med., 122:785, 1965.
5. Roholt, O., Radzimski, G., and Pressman, D., J. Exp. Med., 125:191, 1967.
6. Olins, D.E., and Edelman, G.M., J. Exp. Med., 119:789, 1964.
7. Grey, H., and Mannik, M., J. Exp. Med., 122:619, 1965.
8. Edelman, G.M., Olins, D.E., Gally, J.A., and Zinder, N.D., Proc. Natl. Acad. Sci., 50:753, 1963.
9. Bridges, S.H., and Little, J.R., Biochemistry, 10:2525, 1971.
10. Sher, A., Lord, E., and Cohn, M., J. Immunol. 107:1226, 1971.
11. Klinman, N., J. Immunol. 106:1330, 1971.
12. Poulsen, K., Fraser, K.J., and Haber, E., Proc. Natl. Acad. Sci., 69:2495, 1972.
13. Eisen, H.N., and Siskind, G.W., Biochemistry, 3:996, 1964.
14. Jaquet, H., and Havas, H.F., J. Immunology, 107:1729, 1971.
15. Underdown, B.J., Simms, E.S., and Eisen, H.N., Biochemistry, 10:4539, 1971.
16. Laemmli, U.D., Nature, 227:680, 1970.
17. Sirisinha, S., and Eisen, H.N., Proc. Natl. Acad. Sci., 68:3130, 1971.
18. Porath, J., Axen, R., and Ernback, S., Nature, 214:1302, 1967.
19. Sonoda, S., and Schlamowitz, M., Immunochemistry, 7:885, 1970.
20. Bray, G.A., Anal. Biochem., 1:279, 1960.
21. Nisonoff, A., and Pressman, D., J. Immunol. 80:417, 1958.
22. Karush, F., Adv. Immunol., 2:1, 1962.
23. Eisen, H.N., Simms, E.S., and Potter, M., Biochemistry, 7:4126, 1968.
24. Eisen, H.N., Methods Med. Res., 10:106, 1964.
25. Velick, S.F., Parker, C.W., and Eisen, H.N., Proc. Natl. Acad. Sci., 46: 1470, 1960.
26. Björk, I., and Tanford, C., Biochemistry, 10:1271, 1971.

27. Brient, B.W., Haimovich, J., and Nisonoff, A., Proc. Natl. Acad. Sci., 68: 3136, 1971.
28. Wells, J.V., Fudenberg, H.H., and Givol, D., Proc. Natl. Acad. Sci. 70: 1583, 1973.
29. Painter, R.G., Sage, H.J., and Tanford, C., Biochemistry, 11:1338, 1972.
30. Painter, R.G., Sage, H.J., and Tanford, C., Biochemistry, 11:1327, 1972.
31. Haimovich, J., Givol, D., and Eisen, H.N., Proc. Natl. Acad. Sci., 67: 656, 1970.
32. Eden, A., Lamm, M.E., and Nussenzweig, V., J. Immunology, 108:1605, 1972.
33. Roholt, O.A., Onoue, R., Radzimski, G., and Pressman, D., Immunochemistry, 10:707, 1973.
34. Waterfield, M.D., Prahl, J.W., Hood, L.E., Kindt, T.J., and Krause, R.M., Nature New Biol., 240:215, 1972.
35. Edelman, G.M., Ann. N. Y. Acad. Sci., 190:5, 1971.

